**PCT** WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

**B3**

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| (51) International Patent Classification 7 : A61K 39/00, G01N 33/68, 33/50 | A2 | (11) International Publication Number: **WO 00/45839** |
|---|---|---|
| | | (43) International Publication Date: 10 August 2000 (10.08.00) |

(21) International Application Number: PCT/EP00/00661

(22) International Filing Date: 28 January 2000 (28.01.00)

(30) Priority Data:
A 129/99 1 February 1999 (01.02.99) AT

(71) Applicant *(for all designated States except US)*: CISTEM BIOTECHNOLOGIES GMBH [AT/AT]; Dr. Bohr-Gasse 7b, A-1030 Vienna (AT).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: VON GABAIN, Alexander [AT/AT]; Hockegasse 7, A-1180 Vienna (AT). BUSCHLE, Michael [DE/AT]; Hyrtlstrasse 35/1/1, A-2345 Brunn am Gebirge (AT).

(74) Agents: SONN, Helmut et al.; Riemergasse 14, A-1010 Vienna (AT).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
*Without international search report and to be republished upon receipt of that report.*

(54) Title: A METHOD FOR SELECTING AND PRODUCING VACCINE AND DIAGNOSTIC PREPARATIONS

(57) Abstract

There is disclosed a method for selecting and producing vaccine and diagnostic preparations against a specific pathogenic organism, wherein a non-human vertebrate is immunized with a preparation of the pathogenic organism, samples containing the antibodies to the pathogen-preparation are taken from immunized vertebrates, a gene library of the genome of the pathogenic organism is produced, which gene library is capable of being expressed in host cells, the pathogen polypeptide sequences-expessing gene library-host cells being selectable as regards their binding to an antibody, antigenic polypeptides are identified by contacting the gene library with the antibody-containing sample taken, and those clones of the gene library are selected whose expressed polypeptides bind to an antibody, and the identified antigenic polypeptides are finished to a vaccine or diagnostic preparation.

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Latvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav Republic of Macedonia | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | | | TR | Turkey |
| BG | Bulgaria | HU | Hungary | ML | Mali | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MN | Mongolia | UA | Ukraine |
| BR | Brazil | IL | Israel | MR | Mauritania | UG | Uganda |
| BY | Belarus | IS | Iceland | MW | Malawi | US | United States of America |
| CA | Canada | IT | Italy | MX | Mexico | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NE | Niger | VN | Viet Nam |
| CG | Congo | KE | Kenya | NL | Netherlands | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NO | Norway | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's Republic of Korea | NZ | New Zealand | | |
| CM | Cameroon | | | PL | Poland | | |
| CN | China | KR | Republic of Korea | PT | Portugal | | |
| CU | Cuba | KZ | Kazakstan | RO | Romania | | |
| CZ | Czech Republic | LC | Saint Lucia | RU | Russian Federation | | |
| DE | Germany | LI | Liechtenstein | SD | Sudan | | |
| DK | Denmark | LK | Sri Lanka | SE | Sweden | | |
| EE | Estonia | LR | Liberia | SG | Singapore | | |

# A METHOD FOR SELECTING AND PRODUCING VACCINE AND DIAGNOSTIC PREPARATIONS

The invention relates to a method for selecting and producing vaccine and diagnostic preparations against pathogens of the most varying types.

With the systematic introduction of protective vaccinations, many serious infectious diseases could be repelled in our century. Diseases such as poliomyelitis, tetanus, diphteria, smallpox and meningitis which previously have been fatal have lost their horror by targeted vaccination programs covering complete areas. In 1980, smallpox could even be declared by the WHO to be extinct. With the introduction of recombinant DNA technology it has been possible to develop vaccines which previously have been possible in principle, yet whose broad application could not be realized on acount of the small amount of vaccine material available. Examples thereof are vaccines against the hepatitis B virus and against pneumonia (caused by Bordetella pertussis).

Unfortunately, at present infectious diseases are highly increasing again. On the one hand, this is caused by the fact that ever new, more complex infectious diseases occur, such as, e.g., hepatitis C, AIDS, hemorrhagic intestinal diseases etc., and, on the other hand, by the fact that some pathogens have become resistant to the established vaccines. At the same time, many pathogens which so far could successfully be treated with medicaments, have become resistant to these medicaments. Here, particularly the treatment of pneumonia or meningitis, intestinal infections or diseases like tuberculosis or malaria have to be pointed out. Primarily the increasing resistance of bacterial pathogens to antibiotics poses great problems for world-wide health policies.

Therefore, it seems desirable to provide vaccines effective against these infectious diseases. Unfortunately, finding efficient and safe vaccines is a complex activity. Efficient vaccines mostly are directly derived from the pathogen, e.g. by

chemical or physical inactivation or by attenuation of the pathogen. Such vaccines, however, in principle constitute a safety risk, since an insufficient inactivation may result in actual infection. On the other hand, too drastic an inactivation may lead to denaturing effects which may both reduce the efficiency of the vaccine and have side effects.

In principle, the production of recombinant vaccines is simple, primarily with a view to producing large amounts as are required for a world-wide vaccination campaign, yet the efficiency of the recombinantly prepared vaccine often does not compare to the efficiency of the biogenic vaccine. The reasons for this often reside in the non-nativity of the recombinant vaccines, so that in the vaccinated person not quite exactly those antibodies will be formed which would be required for efficiently combatting an attack by the pathogen.

In the prior art, complex computer-aided modelling methods have been suggested, which should help to optimize recombinant vaccines (Rappuoli et al., Spektrum der Wissenschaft, Spezial 6: Pharmaforschung (1997), pp. 60-69). On account of the fact that more and more knowledge is gained about the genetic basis of the most important pathogens (of numerous pathogens the entire genome has already been completely sequenced), particularly high hopes are placed on this approach. However, the amounts of time and work involved in such a vaccine are enormous, since not only the basic targeted design by molecular modelling is required, but vaccines created on the basis of these calculations often deviate from the predicted binding properties when used in practice and thus at first must be subjected to comprehensive basic tests.

In a further approach it is attempted to first produce an immune response in mice via DNA vaccines which have been created on the basis of predicted ORFs, and to find suitable vaccine candidates via the prediction of HLA-super family-binding T cell epitopes (Hoffman et al, Nature Med. 4 (1998), pp. 1351-1353).

It is thus the object of the present invention to provide a

method for producing vaccines against pathogens with which efficient vaccines can be developed quickly and at low costs. In doing so, the knowledge about the genetic information of the pathogens is to be optimally utilized. Furthermore, vaccines are to be created which can easily be transformed by the immune system of the vaccinated subject into a safe protection against the respective pathogen.

According to the invention, this object is achieved by a method for selecting and producing vaccine and diagnostic preparations against a certain pathogenic organism, which is characterized by the following steps:

- immunizing a non-human vertebrate with a preparation of the pathogenic organism,
- taking samples containing the antibodies to the pathogen-preparation from immunized vertebrates and, optionally, working up these samples,
- producing a gene library of the genome of the pathogenic organism, which gene library is capable of being expressed in host cells, the pathogen polypeptide sequences-expressing gene library-host cells being selectable as regards their binding to an antibody,
- identifying antigenic polypeptides by contacting the gene library with the antibody-containing sample taken, and selecting the gene library for those clones whose expressed polypeptides bind to an antibody,
- isolating and finishing the identified antigenic polypeptides to a vaccine or diagnostic preparation.

With the method according to the invention, it is rapidly and efficiently possible to develop a vaccine or a diagnostic agent against any desired pathogen which optimally leads to a reliable immunization of an organism to be vaccinated. By selecting the gene library of the pathogen for binding to (=recognizing) an antibody whose formation by the (native) pathogen has already been ensured in the infected non-human vertebrate, according to the invention in any case a polypeptide is obtained which naturally is antigenic, its antigenicity being ensured by this selection itself.

The vaccines or diagnostic agents according to the invention can be employed for immunization of or diagnosis in any animal types, yet the preferred application is in humans. Preferred animal pathogens are pathogens for agriculturally useful animals or for pets.

With the diagnostic agents according to the invention, a reliable detection system for infections with a certain pathogen can be provided which - provided with a suitable marker - can be employed for routinely testing organisms potentially infected with this pathogen.

In this manner, polypeptides are obtained with the method according to the invention which are capable of producing antibodies in a vertebrate. For producing the vaccines according to the invention, the polypeptides immediately selected from the gene library can be used, e.g. by purifying them directly by amplification of the host cells and subsequent recovery of the polypeptide from these cells. On the basis of the selected polypeptides or their genetic information, respectively, (which is part of the host cells and thus can be obtained directly), it is, however, also possible to design suitable vaccines. For instance, it is possible to localize the selected polypeptides in the entire genome of the pathogen and to associate them with certain pathogen proteins, particularly if the entire genome has already been sequenced. In principle, this mode of procedure is also possible for pathogens of which there exists only a rough mapping. Then these pathogen proteins may be employed either in their entirety or in truncated form for vaccine production. The parts of the proteins which optionally can be truncated will depend on the polypeptides selected according to the invention which, in the vaccine produced, preferably should remain as complete as possible in their antigenic form. If two or more antigenic polypeptides can be associated with a pathogen protein, the combination of at least these polypeptides frequently can lead to a particularly advantageous vaccine. If the antigenic polypeptides in the pathogen protein are not adjacent, it may also be advisable to provide a spacer between

the polypeptides before the vaccine is finished.

The inventive isolation and finishing of the vaccines or diagnostic agents may thus include the above steps of design adaptation. Isolation may also be effected exclusively on DNA level, which means that the genetic information regarding the antigenic polypeptides from the selected host cells is, e.g., taken over into a further expression vector, is expressed in pharmaceutically suitable expression cells and worked up to a pharmaceutical preparation. Also the vaccine or the diagnostic agent itself can be provided on DNA level; the technology for DNA vaccines is known in principle, and corresponding carrier or administration systems, such as, e.g., by powder-ject injections, have been described.

With the system of the invention, vaccines or diagnostic agents against all infectious pathogens can be developed, i.e., e.g., against bacteria, viruses, fungi, protozoa etc.. The method according to the invention is, however, particularly well suited for pathogens which have a comparatively small genome, which either is easy to sequence or which has already been completely sequenced. Therefore, according to the invention primarily vaccines or diagnostic agents against bacterial or viral pathogens are preferred.

As non-human vertebrates, which according to the invention are infected with the pathogen preparation, all animals are suitable which exhibit sufficient generation of antibodies upon infection with a pathogen, i.e., e.g., amphibians, reptiles, birds or mammals. Preferably animals are used which have already been established in the immunological laboratory, such as frogs (e.g. Xenopus laevis), chickens or rodents, in particular rabbits, rats or mice. According to a preferred embodiment of the present invention, a non-human mammal is immunized with a preparation of the pathogenic organism. In many instances one can depart from the fact that it is advantageous if the test animal is as closely related as possible to the organism which is to receive the vaccine according to the invention, so that the immune reactions occur largely similar.

In the method according to the invention, at first a non-human vertebrate is given a preparation of the selected pathogen so that an immune reaction is triggered in the vertebrate. What is essential is that in this immune reaction which will be termed "immunization" for the purposes of the present invention for the sake of simplicity, antibodies to the pathogen are formed in the vertebrate. Following immunization, these antibodies are taken from the vertebrate and optionally are purified by common methods to a purified antibody preparation. For instance, serum may be taken from the vertebrate from which the antibody preparation used for selection of the gene library will be produced. Immunization of the vertebrates may be carried out with subletal or with letal doses; taking of the antibodies may be effected once, e.g. upon sacrificing the animals, or in several charges, e.g. after several administrations of the pathogen preparation.

Advantageously, the vertebrates are selected such that besides the formation of antibodies, also a formation of antigen-recognizing cells of the cellular system of the immune system is caused by the administration of the pathogen preparation. According to the invention, these cells can then be used for further selection of the polypeptides identified according to the inveniton, e.g. by assaying these peptides or the pathogen proteins associated with these peptides for their binding capacity relative to these cells.

According to a preferred embodiment of the method according to the invention, thus, besides antibody-containing samples, antigen-recognizing cells of the cellular system of the immune system are additionally taken from the immunized vertebrate, with which cells the identified antigenic polypeptides are further selected in that the identified antigenic polypeptides or the complete proteins of the pathogen to be associated with the identified antigenic polypeptides are tested for their capacity of binding to the antigen-recognizing cells, and those polypeptides or proteins are selected for finishing which exhibit a binding capacity to the cells.

For these embodiments, preferably vertebrates are provided in which the cellular components of the immune system are simple to remove and to handle. Among them are particularly birds and animals which with thymus, spleen, bursa and lymphatic nodes have easily removable sources for such cells, the removal of which has long been established. Preferably, T cells are removed as antigen-recognizing cells of the cellular system of the immune system from the immunized vertebrate. According to the invention, thus preferably spleen and lymphatic nodes can be removed from the immunized vertebrate, from which an antigen-recognizing cell-containing suspension is prepared with which the binding capacity of the identified polypeptides or of the associated proteins relative to the antigen-recognizing cells is tested. Removal of T cells or PBMC from peripheral blood is preferred with respect to humans.

In the further selection of the identified antigenic polypeptides, using the antigen-recongizing cells, either the binding capacity of the polypeptides themselves relative to these cells can be assayed, or it may be tested whether certain other regions of the pathogen protein with which the polypeptide can be associated, have a binding capacity relative to these cells. For frequently those regions of the pathogen protein which may trigger a humoral immune response, are not identical with the ones that have a binding capacity for cellular immune response. In doing so, thus parts thereof or the entire pathogen protein with which one or several identified antigenic polypeptides can be associated, may be used as a basis for the binding test relative to antigen-recognizing cells. The selection of the partial sequences thus conveniently is made by means of the known or predicted protein structure such that antigenic motifs will remain as complete as possible. A preferred method thus is characterized in that a combination of partial sequences of the complete pathogen proteins associated with the antigenic polypeptides are used as a basis for the selection regarding the binding capacity.

Advantageously, a pathogen preparation containing the complete

pathogen is used for the introductory infection of the vertebrate. In this manner, the vertebrate can produce an immune response which is completely directed to the native pathogen and thus ensures that the vaccines found according to the invention confer a reliable vaccine protection against the native pathogen.

Frequently, however, the infection of the vertebrate with the entire pathogen is not possible, e.g. if it kills the vertebrate, before a suficient cellular and humoral immune response has occurred, so that a further procedure according to the invention cannot take place. In that case, an inactivated preparation of the pathogen must be started from. In this respect it has been found useful according to the invention to use a homogenisate of the pathogen for initial immunization of the vertebrate. It has been shown that also with this an efficient protection against the native pathogen can be achieved, particularly if care is taken in homogenisation not to treat the pathogens by denaturing methods.

Also fixed pathogenic material (e.g. inactivated or cross-linked material) is usable according to the invention for immunizing the vertebrate. This mode of procedure may be recommendable e.g. for highly infectious pathogens the handling of which is cumbersome and dangerous.

A particularly efficient immune response can be triggered in vertebrates if the pathogen preparation employed for immunizing comprises and adjuvant. Advantageously, organic polycations or a mixture of organic cations is used as adjuvants. Particularly preferred adjuvants are basic polypeptides, in particular polyarginine or polylysine.

As has been mentioned, with the present invention it is possible to produce vaccines and diagnostic agents against a plurality of pathogens. Preferably, however, vaccines against human pathogens are provided for which there exists no or only an insufficient immunization alternative and/or which have developed resistances to available chemical or immunological treatments. Preferred

pathogens which are to be fought within the scope of the present invention may thus be selected from the group of Borrelia burgdorferi, Chlamydia spp., group A streptococci, non-typifyable haemophilus influenzae, Legionella pneumophila, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Staphylococcus aureus, Pseudomonas aeruginosa, Rickettsia rickettsii, Shigella spp., Toxoplasma gondi or Treponema pallidum.

Further, preferred pathogens are pathogenic Aspergillus, Candida, Mycobacterium, Rhizopus, Trichophyton, Microsporum, Trypanosoma, Pneumocystis, Plasmodium, Meningococcus and Chlostridia strains as well as retroviruses or hematogenic viruses.

Since in the cellular recognition of the pathogenic proteins, the exact native spatial structure is not of importance, polypeptides which have been produced with a polypeptide sequencer are preferably used as a basis for testing the binding capacity relative to the antigen-recongizing cells.

The choice of the gene library or of the host cells may be arbitrary; what is essential is that the gene library host cells are selected on the basis of the binding of the expressed polypeptides to the antibodies taken from the vertebrates. Accordingly, for the sake of simplicity, the expressed polypeptides should be presented on the outer side of the cells, e.g. as a portion of the outer domain of a membrane protein. The length of the expressed polypeptides is then chosen by way of the respective requiremetns of the gene library/host cell system, yet frequently it ranges between 10 and 200 amino acids, in particular between 20 and 100 amino acids. The selection of the cells may be effected e.g. via a system in which the binding site for a selection agent or a transport channel for the selection agent is blocked by the binding of the antibody to the expressed polypeptide. A particularly advantageous system for such a selection has been described in WO 99/30151 A the contents of which herewith is included in the present application. Preferably, thus, the expressed polypeptides are outwardly presented as a part of the membrane protein of the

gene library host cells. Furthermore, a selection system is preferred in which the gene library host cells become resistant to a selection agent because of the binding of an antibody to the expressed polypeptide.

In this preferred system, those embodiments in which the selection is achieved by a viral agent which is pathogenic for the gene library host cells have proven particularly useful. In this case, the virus receptor in the cells may be constructed such that the former is blocked by the binding of the antibody to the expressed polypeptide, e.g. by intermolecular interaction or by steric blocking. Preferably, such gene library host cells comprise an OmpA, LamB or FhuA selection system.

If one or several polypeptides which bind to one of the antibodies formed in the vertebrate have been identified by the gene library selection, the former can be localized in the genome of the pathogen and associated with certain pathogen proteins (or ORFs). It is, of course, a prerequisite that at least a rough gene mapping of the pathogens is already available. It is, however, particularly advantageous if the complete gene sequence of the pathogen is already available. Accordingly, by using a genomic analysis of the pathogenic organism as a basis, preferably the selected antigenic polypeptides are associated to defined proteins of this pathogenic organism, and those antigenic polypeptides which have been associated with the same protein, are combined.

These polypeptide combinations can then be used as a basis for the binding test relative to antigen-recognizing cells, optionally in combination with further partial sequences of the associated pathogen protein (or also as entire protein).

Determination of the binding properties may be effected with a plurality of assays. The ones most commonly used at present are described in Romero et al (Mol. Med. Today 4, (1998), pp. 305-312), among them also the Elisspot assay by which T cells which bind to an (MHC-II-presented) antigen due to their cytokine secretion (mostly interferon-γ) can be identified. This assay

not only is established, standardizable and simple to handle, it is also extremely sensitive for the purposes of the present invention, particularly since for this assay no cellular *in vitro* proliferation is required. According to a preferred embodiment of the method according to the invention, thus the binding capacity relative to the withdrawn antigen-recognizing cells is determined by using the Elisspot assay.

Preferably, the finishing of the vaccines and diagnostic agents of the invention comprises admixing a pharmaceutically acceptable carrier as well as further auxiliary components.

For the subject matter of the present invention, initial immunization is not restricted to non-human vertebrates. According to a preferred variant of the subject matter of the present invention, both the antibodies and also optionally the antigen-recognizing cells are recovered from humans and are subjected to the selection and production methods according to the invention. In this instance, primary immunization is, however, either left up to nature, i.e. antibodies and T cells are taken from already immunized patients, or it is worked with non-infectious pathogen preparations. Especially for the preparation of efficient vaccines against human pathogens, the withdrawal of antibodies and T cells from humans who have already successfully overcome an infection with this pathogen and have built up a sufficient cellular and humoral protection is a particularly preferred variant of the present invention.

The present invention thus also relates to a method variant in which the step of "immunizing a non-human vertebrate", is replaced by the method step "selecting a person who has overcome an infection with that particular pathogen and has formed antibodies as well as antigen-recognizing cells". When drawing antibodies or antibody-recognizing cells from humans, of course, care must be taken that such withdrawal is effected according to established methods of human medicine and does not have any grave consequences for the donating person. From the above it results that such a method will constitute neither a therapeutic, nor a diagnostic nor a surgical method for the

donating person, since this donating person already counts as immunized and does not obtain any therapeutic effect by this withdrawal. Nevertheless, this "humanized" realization of the present invention for recognizing peptide epitopes is an important aspect of the present invention.

According to a further aspect, the present invention relates to a vaccine preparation comprising a selected polypeptide or a protein containing this polypeptide or the DNA thereof, produced or obtainable by the method according to the invention.

Furthermore, the present invention relates to a diagnostic preparation comprising a selected polypeptide, prepared or obtainable by the method of the invention, which comprises a marker for detecting the polypeptide.

As markers which can be used for detecting the polypeptide, all those usable for detecting the binding of two substances are under consideration, preferably, however, radioactive, fluorescent, chromogenic or amplifyable markers are coupled to the diagnostic agent according to the invention. Just like vaccination, diagnosis may take place on nucleic acid level. A further aspect of the present invention thus relates to a diagnosis kit, comprising a diagnostic preparation according to the invention and detection reagents for the marker.

According to a further aspect, the present invention relates to a kit for carrying out the method of the invention, comprising

- a gene library of the genome of the pathogenic organism,
- host cells in which the gene library can be expressed, wherein the pathogen polypeptide sequences-expressing gene library host cells are selectable with a view to their binding to an antibody, and
- a selection agent.

The potential of the antigen selection of the present invention is, however, not restricted to vaccines and diagnostic agents against certain pathogens. It has been shown that the method according to the invention is also suitable for preparing

efficient tumor antigens. Particularly with the combination of humoral and cellular selection which is preferred according to the invention, particularly suitable tumor antigens can be found. With the tumor antigens found according to the invention, particularly effetive tumor vaccinations can be carried out. However, instead of immunizing the non-human vertebrate with the pathogen preparation, the vertebrate is immunized with a preparation of the respective tumor cell, or antibodies and optionally antigen-recognizing cells are taken from a human tumor patient.

The invention will be explained in more detail by way of the following schematic example as well as the drawing figures to which, of course, it is not restricted.

Fig. 1 (schematically) shows the method according to the invention for identifying antigens; Fig. 2 shows the construction of a gene library for a microbial genome; Figs. 3 and 4 show the selection of the antibody-binding cells by OmpA or LamB selection; Figs. 5 and 6 show the association of the identified polypeptides with the pathogen proteins via genomics; Fig. 7 shows the generation of overlapping peptide fragments for further characterization by means of T cell binding assay; and Fig. 8 shows the screening of the antigen-binding polypeptide by means of Elisspot assay.

Example:

A mouse is immunized with a homogenized pathogenic microbe, polyarginine having been admixed to the homogenisate. After immunization has taken place, serum is taken from the mouse, and spleen and lymphatic nodes are removed. The serum is used for identifying antigens which are recognized by the antibodies in the serum; from spleen and lymphatic nodes, a suspensioin of T cells is prepared (Fig. 1).

An expression gene library is prepared according to WO 99/30151 A, wherein the microbial genome is digested to fragments of a length of approximately 150 bp and is inserted

into suitable selection/expression vectors. With these expression vectors, host cells are transformed, a selectable gene library being generated (Fig. 2).

The antibodies withdrawn from the mouse are admixed with the genetic library host cells, the antibodies being able to bind to suitable expressed structures (Fig. 3). Fig. 4 shows a system in which selection is effected with phages. Binding of the antibody to the expressed polypeptide at the outside of the cell results in a steric blocking of the phage binding site. By this, the cell cannot be infected with the normally letal phage and is positively selected, resulting in an enrichment (e.g. by the factor $10^3$ to $10^6$; higher or lower enrichments depending on the selection system) of those clones which comprise antigenic microbial peptides.

On the basis of the sequence of the expressed polypeptide, the localization of the polypeptide in the pathogen genome is carried out via a genomic data library of the microbial pathogen, and the polypeptide is associated with a certain protein or ORF (Fig. 5). Since humoral and cellular antigens often are separate structures on a protein (Fig. 6), overlapping peptide fragments of the total protein sequence found via the genomic data library are generated, e.g. by automated peptide synthesizers (Fig. 7).

The identified antigenic polypeptides and the generated samples are assayed for their binding to T cells by means of an Elisspot assay. In doing so, the polypeptide samples are provided in a suitable reaction vessel, e.g. a microtiter plate coated with a cytokine-specific antibody, and admixed with the spleen and lymphatic node suspension from the mouse. T cells which bind to an antigen (which, e.g., is presented to them by MHC-II-carrying antigen presenting cells) produce - upon binding to the antigen - cytokines, e.g. interferon-γ. The latter binds to the immobilized antibody and may be detected e.g. by a marked secundary antibody. Those wells in the microtiter plate in which T cell binding has taken place exhibit a positive cytokine reaction.

It has been shown that with the present invention, a particularly efficient high-throughput method for finding and producing suitable, highly immunogenic vaccines and reliable diagnostic agents for infections with pathogens is provided.

With this system, entire genomes of pathogenic organisms can be searched for their particularly antigenic proteins or polypeptide sequences within a short time, whereby suitable antigenic vaccine candidates can immediately be found for practical application.

Claims:

1. A method for selecting and producing vaccine and diagnostic preparations against a certain pathogenic organism, characterized by the following steps:

- immunizing a non-human vertebrate with a preparation of the pathogenic organism,
- taking samples containing the antibodies to the pathogen-preparation from immunized vertebrates and, optionally, working up these samples,
- producing a gene library of the genome of the pathogenic organism, which gene library is capable of being expressed in host cells, the pathogen polypeptide sequences-expressing gene library-host cells being selectable as regards their binding to an antibody,
- identifying antigenic polypeptides by contacting the gene library with the antibody-containing sample taken, and selecting the gene library for those clones whose expressed polypeptides bind to an antibody,
- isolating and finishing the identified antigenic polypeptides to a vaccine or diagnostic preparation.

2. A method according to claim 1, characterized in that the pathogen is a bacterial or viral pathogen.

3. A method according to claim 1 or 2, characterized in that a non-human mammal is immunized with a preparation of the pathogenic organism.

4. A method according to any one of claims 1 to 3, characterized in that besides antibody-containing samples, additionally antigen-recognizing cells of the cellular system of the immune system are withdrawn from the immunized vertebrate, by means of which the identified antigenic polypeptides are furter selected by testing the identified antigenic polypeptides or the complete proteins of the pathogen to be associated with the identified antigenic polypeptides for their binding capacity to antigen-recognizing cells and selecting those polypeptides or proteins for finishing which exhibit a binding capacity to the

cells.

5. A method according to claim 4, characterized in that T cells are withdrawn from the immunized vertebrate as antigen-recognizing cells of the cellular system of the immune system.

6. A method according to claim 4 or 5, characterized in that a combination of partial sequences of the complete pathogen proteins associated with the antigenic polypeptides are used as a basis for the selection regarding the binding capacity.

7. A method according to any one of claims 1 to 6, characterized in that the pathogen preparation employed for immunizing contains the complete pathogen.

8. A method according to any one of claims 1 to 6, characterized in that the pathogen preparation employed for immunizing comprises a homogenisate of the pathogen.

9. A method according to any one of claims 1 to 8, characterized in that the pathogen preparation employed for immunizing comprises an adjuvant.

10. A method according to claim 9, characterized in that organic polycations or a mixture of organic cations is employed as adjuvant.

11. A method according to claim 10, characterized in that basic polypeptides, in particular polyarginine or polylysine, are used as adjuvant.

12. A method according to any one of claims 1 to 11, characterized in that the pathogens are selected from the group of Borrelia burgdorferi, Chlamydia spp., group A streptococci, non-typifyable Haemophilus influenzae, Legionella pneumophila, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Staphylococcus aureus, Pseudomonas aeruginosa, Rickettsia rickettsii, Shigella spp., Toxoplasma gondi or Treponema pallidum.

13. A method according to any one of claims 1 to 12, characterized in that a rodent, in particular a rabbit, a mouse or a rat, is immunized.

14. A method according to any one of claims 1 to 13, characterized in that the test for binding capacity relative to the antigen-recognizing cells is based on polypeptides which have been produced with a polypeptide sequencer.

15. A method according to any one of claims 1 to 14, characterized in that serum is taken from the immunized vertebrate from which an antibody preparation is produced with which the gene library is selected.

16. A method according to any one of claims 1 to 15, characterized in that spleen and lymphatic nodes are removed from the immunized vertebrate from which an antigen-recognizing cell-containing suspension is prepared with which the binding capacity of the indentified polypeptides or the associated proteins relative to the antigen-recongizing cells is tested.

17. A method according to any one of claims 1 to 16, characterized in that the expressed polypeptides are outwardly presented as a part of a membrane protein of the gene library host cells.

18. A method according to any one of claims 1 to 17, characterized in that the gene library host cells become resistant to a selection agent due to the binding of an antibody to the expressed polypeptide.

19. A method according to any one of claims 1 to 18, characterized in that the gene library host cells comprise an OmpA, LamB or FhuA selection system.

20. A method according to any one of claims 1 to 19, characterized in that the selected antigenic polypeptides are associated to defined proteins of a pathogenic organism by using a genomic analysis of this pathogenic organism as a basis, and

those antigenic polypeptides which have been associated to the same protein are combined.

21. A method according to any one of claims 1 to 20, characterized in that the binding capacity relative to the withdrawn antigen-recognizing cells is determined by using the Elisspot assay.

22. A method according to any one of claims 1 to 21, characterized in that processing of the selected polypeptides to vaccines or diagnostic agents comprises admixing a pharmaceutically acceptable carrier.

23. A method for selecting and producing vaccine and diagnostic preparations against a certain pathogenic organism, in particular against a bacterial or viral pathogen, characterized by the following steps:

- selecting a donor person who has overcome an infection with that specific pathogen and has formed antibodies as well as possibly antigen-recognizing cells,
- taking samples containing antibodies to the pathogen preparation from the donating person, and optionally working up these samples,
- preparing a gene library of the genome of the pathogenic organism, which gene library is capable of being expressed in host cells, the gene library host cells expressing the pathogen polypeptide sequences being selectable regarding their binding to an antibody,
- identifying antigenic polypeptides by contacting the gene library with the antibody-containing sample taken, and selecting the gene library for those clones whose expressed polypeptides bind to an antibody,
- isolating and finishing the identified antigenic polypeptides to a vaccine or diagnostic preparation.

24. A method according to claim 23, characterized in that it is proceeded according to any one of claims 4 to 12 and 14 to 22, the donor person being the immunized vertebrate.

25. A vaccine preparation comprising a selected polypeptide, produced by a method according to any one of claims 1 to 24.

26. A dignostic preparation comprising a selected polypeptide, produced by a method according to any one of claims 1 to 24, comprising a marker for detecting the polypeptide.

27. A diagnosis kit, comprising a preparation according to claim 26 and detection reagents for the marker.

28. A kit for carrying out the method according to any one of claims 1 to 24, comprising

- a gene library of the genome of the pathogenic organism,
- host cells in which the gene library can be expressed, the pathogen polypeptide sequences-expressing gene library-host cells being selectable with a view to their binding to an antibody, and
- a selection agent.

29. The use of a method according to any one of claims 1 to 24 for preparing a tumor antigen.

FIG. 1

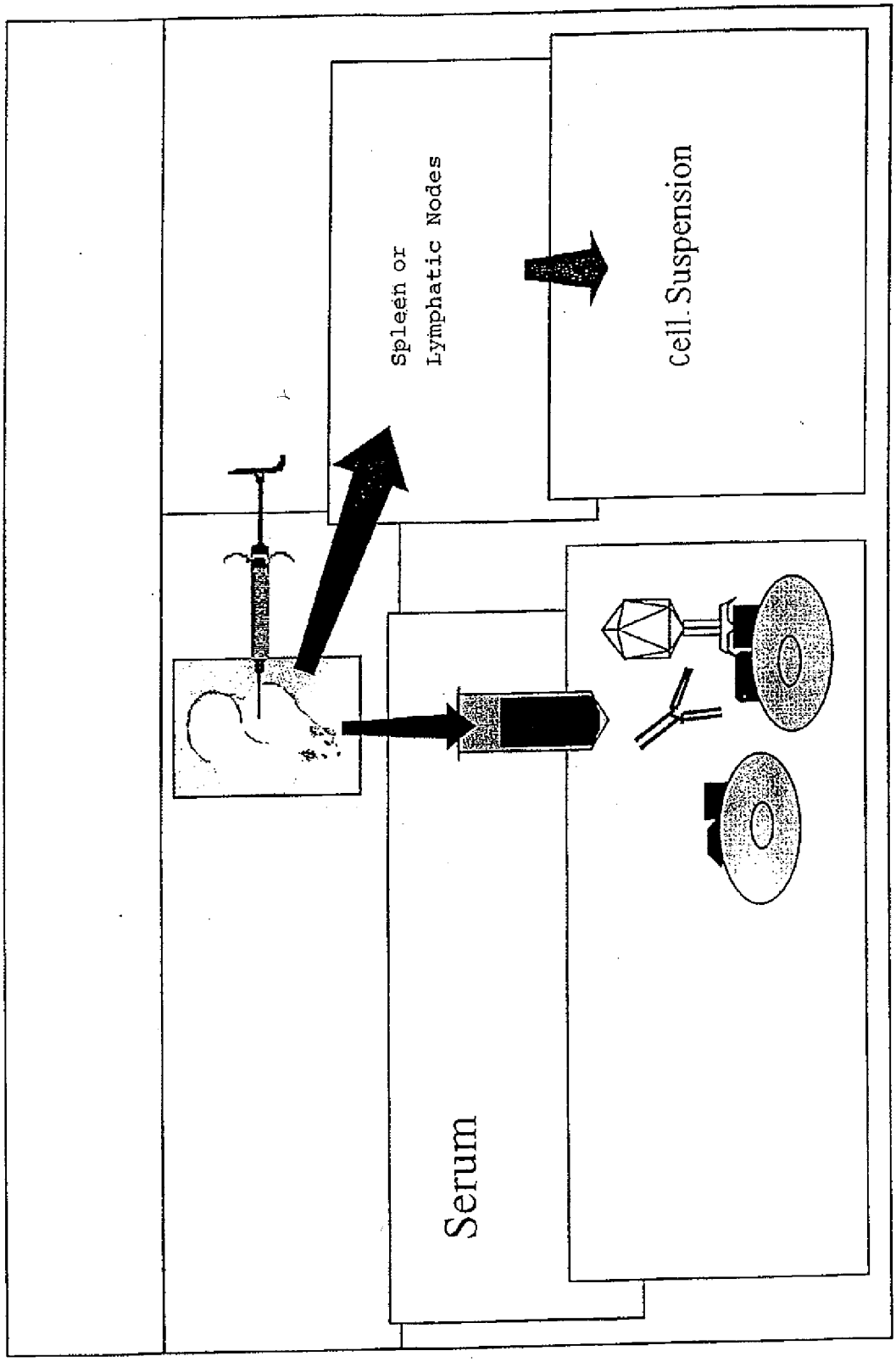


FIG. 2

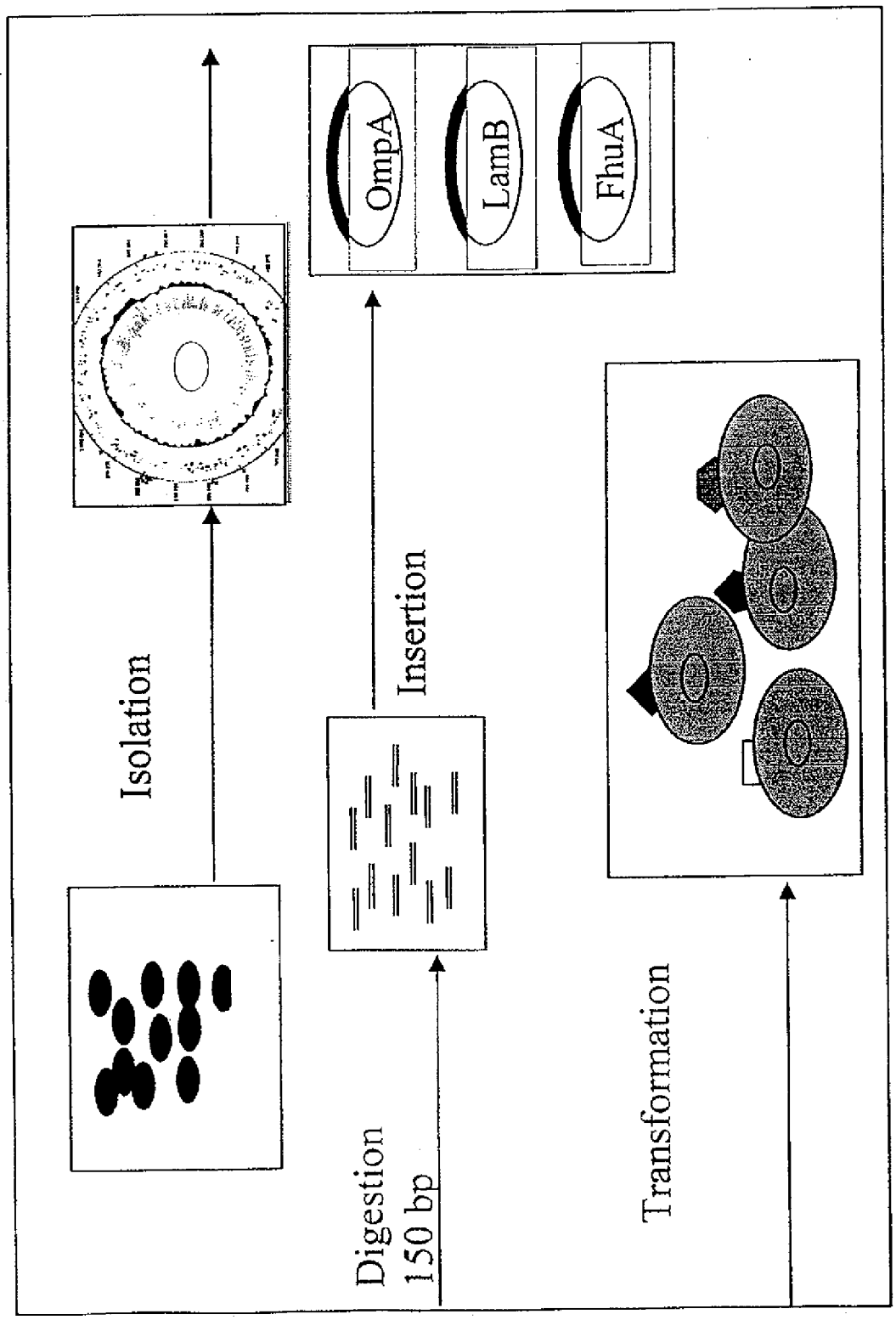

OmpA
LamB
FhuA
Isolation
Insertion
Digestion
150 bp
Transformation

FIG. 3

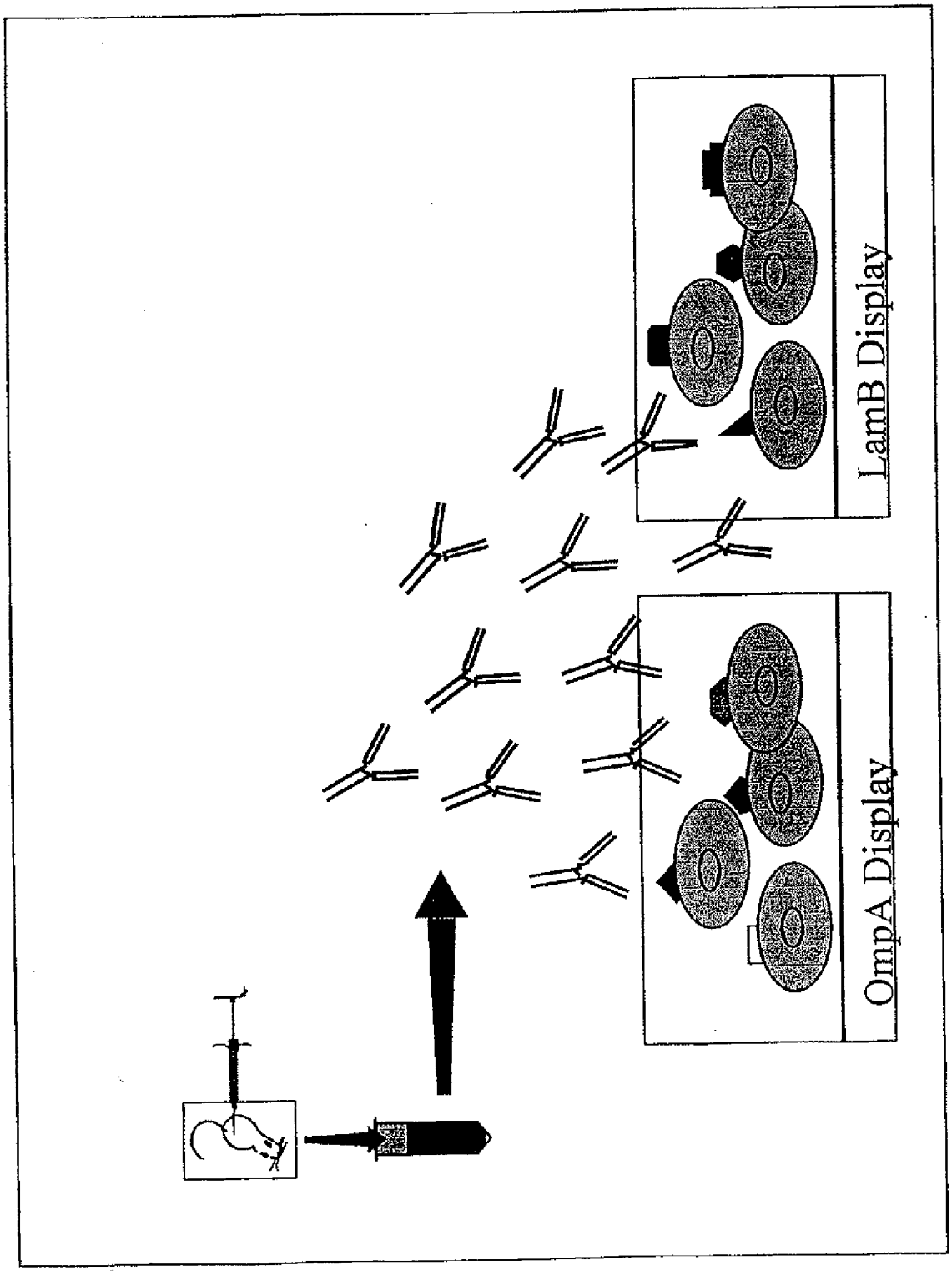


FIG. 4

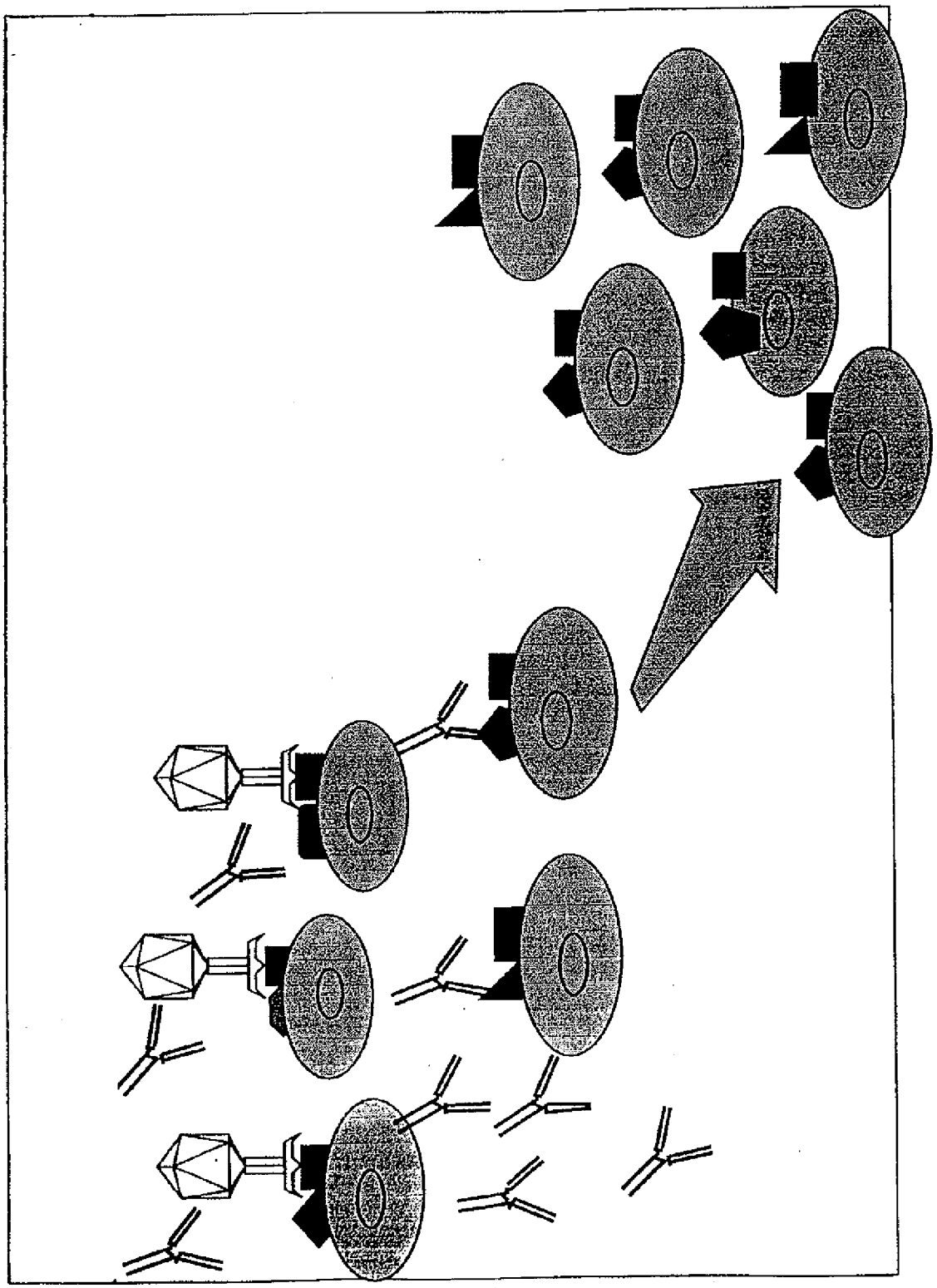


FIG. 5

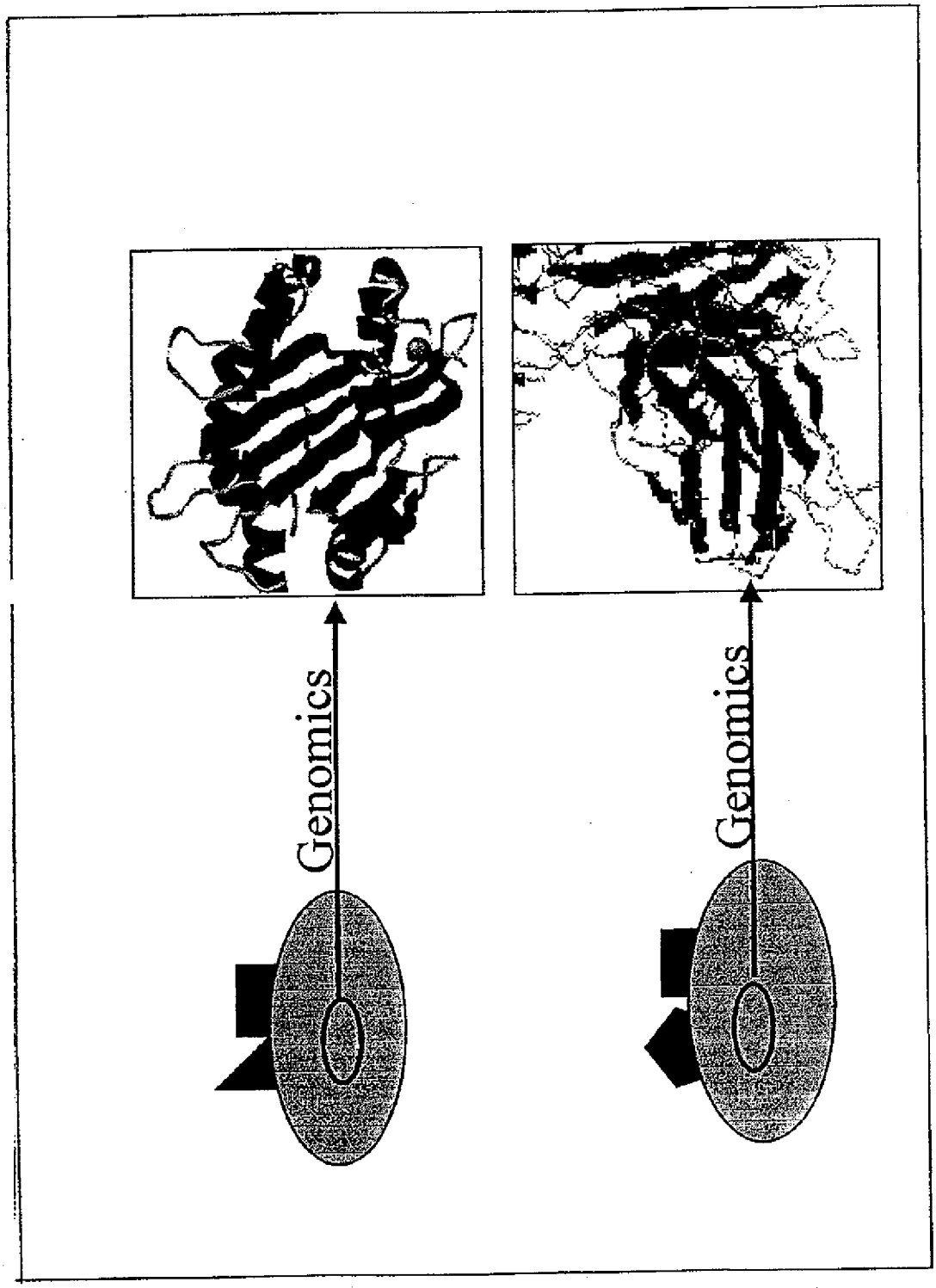


FIG. 6

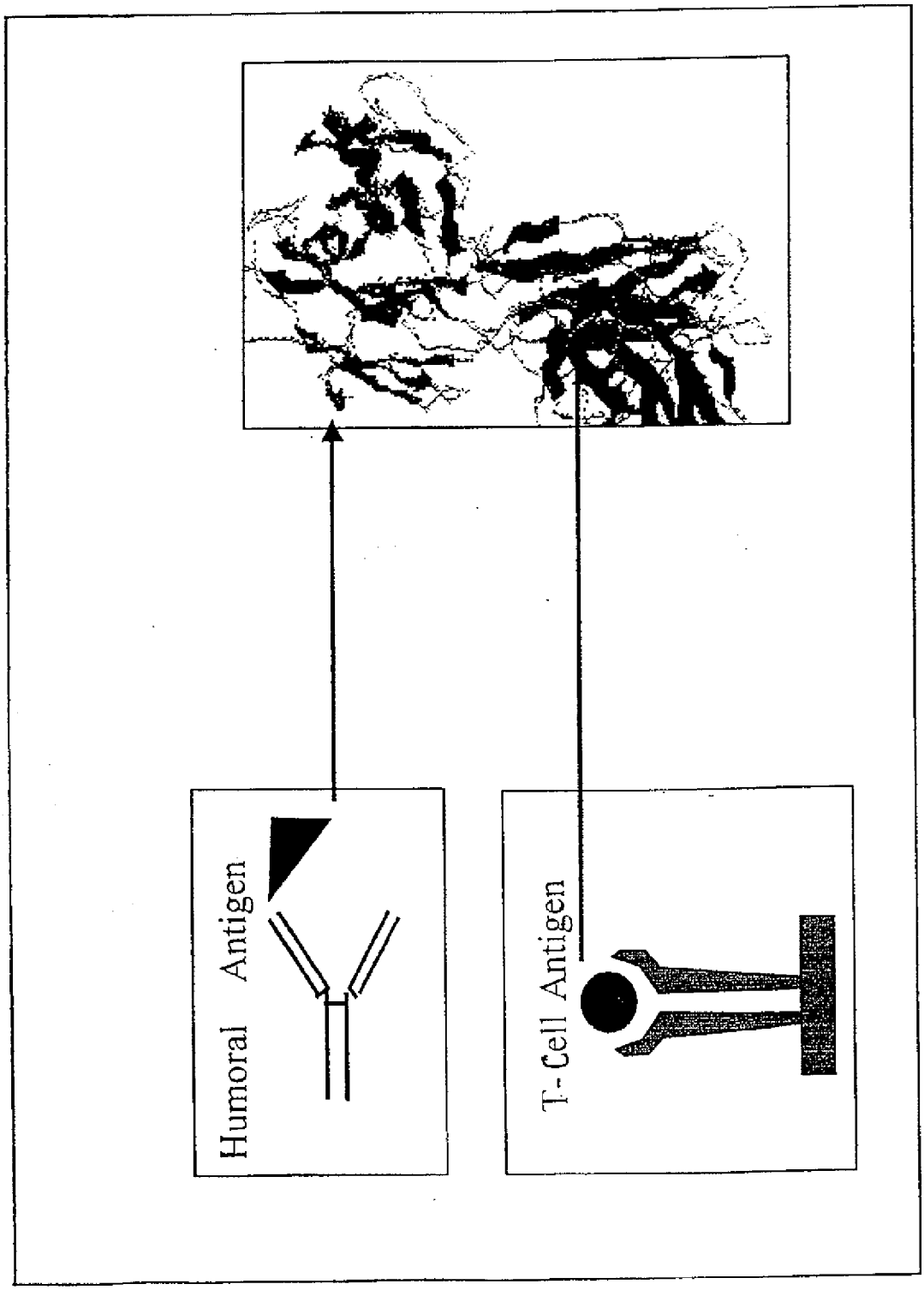


FIG. 7

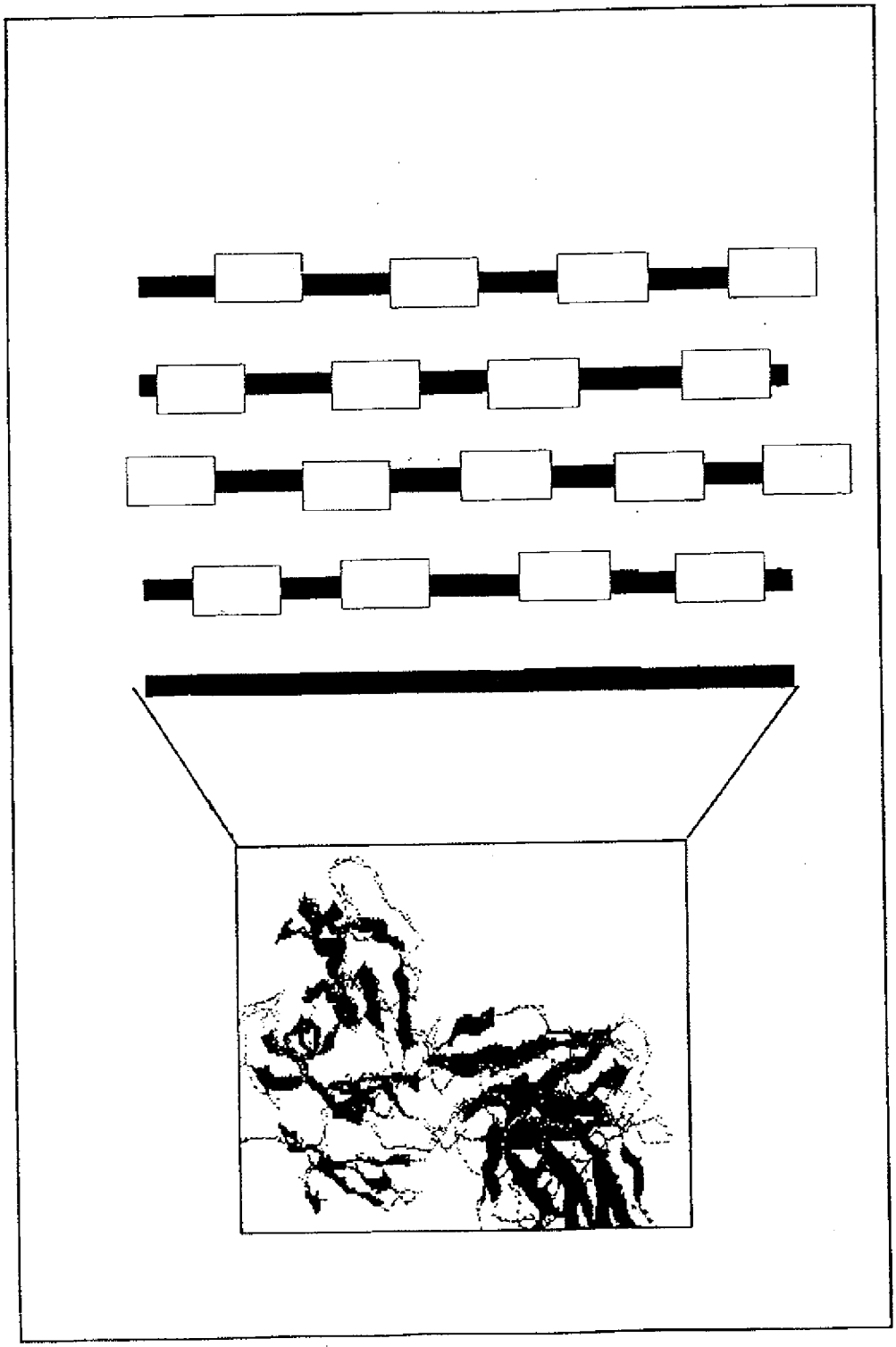
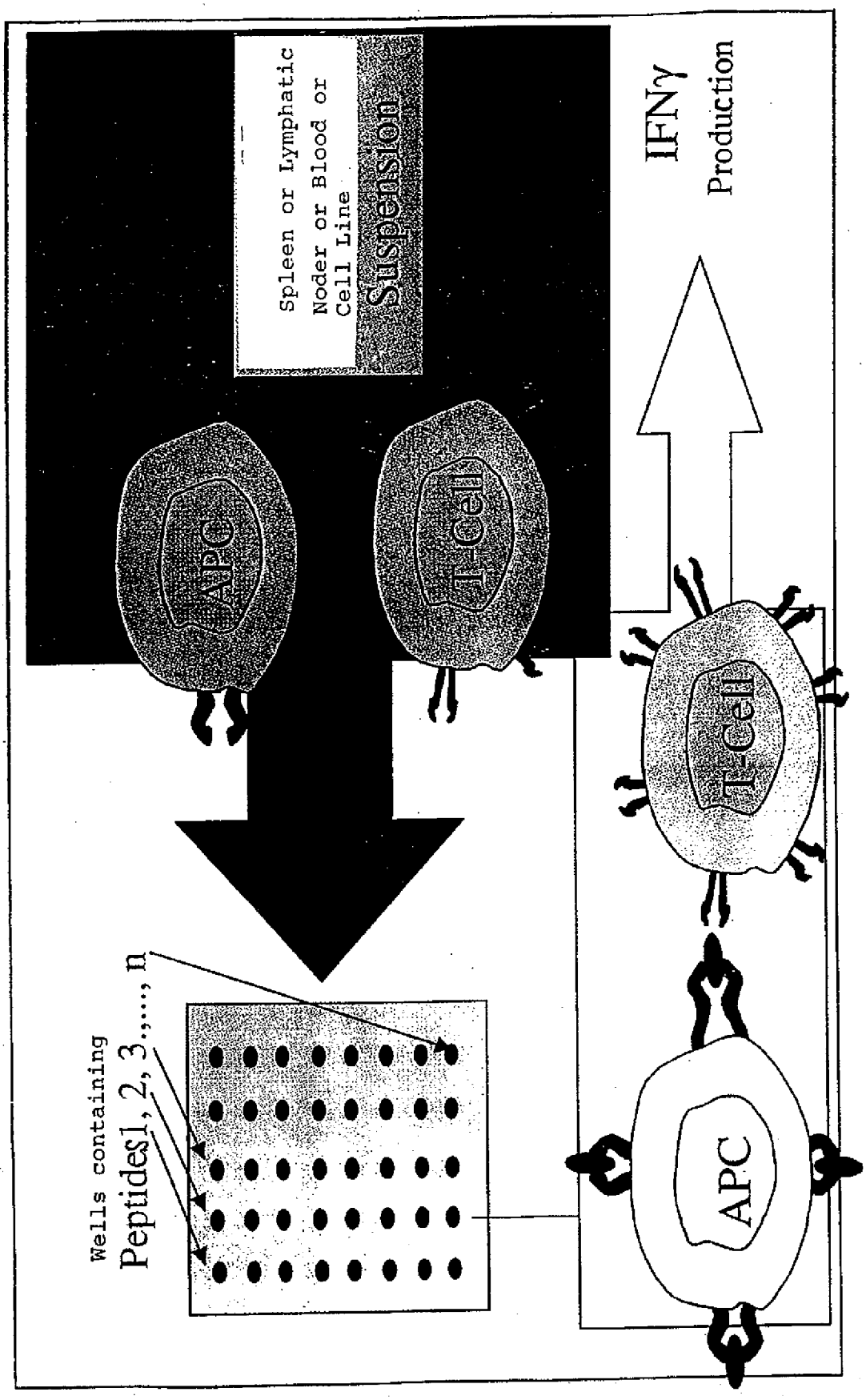


FIG. 8


Spleen or Lymphatic Noder or Blood or Cell Line
Suspension
APC
T-Cell
IFNγ Production
T-Cell
APC
Wells containing Peptides 1, 2, 3,..., n